ENDOTHELIAL FUNCTION

Vascular endothelial function and circulating endothelial progenitor cells in patients with cardiac syndrome X

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Background: Endothelial dysfunction and microvascular abnormalities have been reported in patients with cardiac syndrome X (CSX), but the underlying mechanisms are unclear. Recent insights suggest that the injured endothelial monolayer is regenerated by circulating bone marrow-derived endothelial progenitor cells (EPCs).

Aim: To test the hypothesis that the biology of altered EPCs might contribute to the pathophysiology of CSX.

Methods: 34 subjects (mean (SD) age: 62 (7) years) were enrolled in the study, including 12 patients with CSX, 12 stable subjects with coronary artery disease (CAD) and 10 healthy controls. The number and adhesive function of EPCs were measured in peripheral-blood samples from these study participants.

Results: The baseline characteristics in patients with CSX and CAD were enhanced Framingham risk scores, more hypertension and lower high-density lipoproteins than the controls. Patients with CSX and CAD had significantly decreased endothelium-dependent flow-mediated vasodilation (FMD) compared with normal controls (normal controls vs CSX vs CAD: 10.6% (3.5%) vs 6.1% (1.8%) vs 4.1% (1.9%), $p<0.001$), but the difference was not found in endothelium-independent nitroglycerine-mediated vasodilation ($p=0.159$). Reduced numbers of colony-forming units (CFU) of EPCs were noted in patients with CSX and CAD (normal vs CSX vs CAD: 41 (9) vs 30 (7) vs 14 (7) CFU/well, $p<0.001$). Levels of EPCs were shown to be associated with FMD ($r=0.557$, $p=0.001$) and high-density lipoprotein ($r=0.339$, $p=0.049$). Also, attenuated fibronectin adhesion function of EPCs was found in patients with CSX and CAD compared with normal subjects (104 (12) vs 80 (20) vs 65 (13)/well, $p<0.001$).

Conclusions: This study clearly showed for the first time that compared with normal subjects, patients with CSX have decreased levels and adhesive function of circulating EPCs. These findings may explain the underlying mechanisms which contribute to the endothelial dysfunction and microvascular abnormalities observed in patients with CSX.

About 30% of patients undergoing coronary angiography for angina have been found to have normal epicardial coronary arteries.[1] A subgroup of these patients having typical exertional chest pain associated with ischaemic-like ST segment changes on exercise testing and with no evidence of epicardial coronary vasospasm are diagnosed as having cardiac syndrome X (CSX).[2] Although CSX has been recognised for more than three decades, it remains underappreciated as a distinct clinical entity.[3] Multiple pathophysiological abnormalities have been proposed in patients with CSX, including reduced coronary flow reserve, abnormal pain perception, endothelial dysfunction, altered adrenergic activity, parasympathetic impairment and increased platelet aggregability.[4–9] Convincing evidence indicated that microvascular ischaemia secondary to endothelial dysfunction is a leading pathophysiological explanation for CSX.[6]

The endothelium lines the vasculature lumen and is a multifunctional organ system that affects vascular tone, smooth muscle cell proliferation, platelet aggregation, monocyte and leucocyte adhesion. Endothelial dysfunction leads to impaired control of vascular tone, a decrease in the release of anti-inflammatory factors and reduced availability of nitric oxide.[10] Some studies indicated that endothelial function in patients with CSX has been shown to be similarly impaired as in patients with established coronary artery disease (CAD).[11] The loss of intact endothelial integrity and function sets in motion a cascade of serial events that lead to atherosclerosis and cardiovascular complications.[12]

Increasing evidence suggests that the injured endothelial monolayer may be regenerated by circulating bone marrow-derived endothelial progenitor cells (EPCs), which accelerates re-endothelialisation and protects against the initiation of atherosclerosis.[13 14] Levels of circulating EPCs have been shown to be associated with endothelial function and cardiovascular risk factors.[15 16] Decreased levels of circulating EPCs independently predict cardiovascular events, suggesting an important role for endogenous vascular repair of EPCs in modulating the clinical course of CAD.[17 18] However, no previous study has mentioned the role of circulating EPCs in patients with CSX. Here, we measured the number and adhesive function of circulating EPCs in patients with CSX, and tested the hypothesis that altered EPC biology might contribute to the pathophysiology of CSX.

METHODS

Patients and controls

We evaluated 34 participants (mean (SD) age: 62 (7) years; 17 females), including 12 patients with CSX, 12 subjects with stable CAD and 10 healthy controls. All subjects underwent clinical and risk factor assessment, baseline 12-lead ECG, and M-mode and two-dimensional echocardiography.

All 12 consecutive subjects with CSX fulfilled the criteria for CSX, including the typical exertional chest pain, a positive treadmill exercise test (non-upsloping ST segment >2 mm in chest leads or >1 mm in limb leads), angiographically normal

Abbreviations: CAD, coronary artery disease; CFUs, colony-forming units; CSX, cardiac syndrome X; EPCs, endothelial progenitor cells; FMD, flow-mediated vasodilation; hsCRP, high-sensitive C reactive protein; NMD, nitroglycerine-mediated vasodilation; TNFα, tumour necrosis factor α

coronary arteries in the absence of coronary artery spasm, and normal regional and global left ventricular function at rest assessed by M-mode and two-dimensional echocardiography. Patients suffering from vasospastic angina, hypertensive heart disease with left ventricular hypertrophy, valvular heart disease (mitral valve prolapse, moderate to severe aortic regurgitation and mitral regurgitation), left ventricular dysfunction (left ventricular ejection fraction $\leqslant$50%), congenital heart disease, diabetes mellitus, and significant endocrine, hepatic (total bilirubin $>$1.6 mg/dl) or renal diseases (serum creatinine $>$2.0 mg/dl) were excluded from the study. Non-cardiac causes of chest pain, such as gastrointestinal and musculoskeletal disorders, were investigated and excluded as appropriate. All subjects with smoking history (current or past personal history) were excluded from this study, and no female participant underwent hormone replacement therapy.

The control group included 10 subjects who had experienced atypical chest discomfort and proved negative on treadmill exercise test. Coronary angiography was not performed in the control group due to ethical consideration. Another 12 chronic stable patients with CAD were also enrolled in the study; all of these patients had documented CAD on coronary angiogram (at least one lesion with $>$50% stenosis in luminal diameter on coronary angiogram), and none of these patients had angina symptoms for at least 1 month. All patients gave informed consent, and the study was approved by the local research ethics committee. The protocols of this study were consistent with ethical guidelines provided in the 1975 Helsinki Declaration.

Isolation of EPCs and colony-forming assay

A 20 ml sample of venous blood was used for the isolation of endothelial progenitor cells. All samples were processed within 2 h after collection, and peripheral-blood mononuclear cells were isolated by Histopaque-1077 (1.077 g/ml, Sigma, St. Louis, Missouri, USA) density-gradient centrifugation. After centrifugation, the mononuclear cells were carefully collected into a 50 ml polypropylene tube. Recovered cells were washed twice with phosphate-buffered saline and 20% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). Isolated cells were subsequently resuspended in growth medium (EndoCult liquid medium, StemCell Technologies, Vancouver, Canada), and a total of 5×10^6 peripheral-blood mononuclear cells were preplated in a fibronectin-coated six-well plate in duplicate. After 48 h, the non-adherent cells were collected by pipetting the medium in each well up and down three times, and 1×10^6 cells were replated onto a fibronectin-coated 24-well plate. On day 5 of the assay, the number of colony-forming units (CFUs) per well for each sample was counted. A colony of EPCs was defined as a central core of round cells with elongated sprouting cells at the periphery, as shown in fig 1A. A central cluster alone without associated emerging cells was not counted as a colony. All colonies were counted manually in a minimum of three wells by two independent observers who were unaware of the patients' clinical profiles. To assess reproducibility, we also determined the CFU twice in two separate blood samples from six participants (two subjects in each groups) at least 2 weeks apart. The interobserver correlation was 0.92, whereas the intraclass correlation, obtained by a single observer who analysed two blood samples obtained at least 2 weeks apart from a single subject, was 0.87.

Confirmation of CFU phenotype

Colonies were assessed for endothelial cell markers on day 7. Endothelial cell lineage was further confirmed by indirect immunostaining with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-acetylated low-density lipoprotein (DiI-acLDL; Molecular Probes) and costaining with Bandeiraea simplicifolia lectin I (Sigma, fig 1B,C). Briefly, the adherent cells were first incubated with 2.4 μg/ml DiI-acLDL for 1 h and then fixed in 2% paraformaldehyde and counterstained with 10 μg/ml FITC-labelled lectin from Ulex europaeus (UEA-1; Sigma). The adherent cell population was also characterised by immunofluorescence staining for the expression of VE-cadherin, platelet–endothelial cell adhesion molecule-1 (CD-31) and von Willebrand factor. The fluorescent images were recorded under a laser scanning confocal microscope.

Fibronectin adhesion assay of EPCs

EPCs (day 7) from all subjects were washed with phosphate-buffered saline and gently detached with 0.5 mmol/l EDTA in phosphate-buffered saline. After centrifugation and resuspension in basal medium with 5% fetal bovine serum, identical cells were placed on a fibronectin-coated six-well plate and incubated for 30 min at 37℃. Gentle washing with phosphate-buffered saline three times was performed after 30 min adhesion, and adherent cells were counted by independent blinded investigators. As shown in fig 1D–F, phenotyping of endothelial characteristics of adherent cells by indirect immunostaining was performed with Dil-acLDL and BS-1 lectin.

Endothelium-dependent flow-mediated vasodilation and endothelium-independent nitroglycerine-mediated vasodilation

Endothelium-dependent flow-mediated vasodilation (FMD) was assessed using a 7.5-MHz linear array transducer (Hewlett-Packard Sonos 5500, Andover, Massachusetts, USA) to scan the brachial artery in longitudinal section as described previously.[19] Briefly, all subjects were asked to fast and withhold all medications for 24 h before the endothelial function test. To minimise mental stress, care was taken to make the patients as comfortable as possible, and the procedure was carried out in a quiet air-conditioned room (25℃). The left arm was stabilised with a cushion, and a sphygmomanometric cuff was placed on the forearm. A baseline image was acquired, and blood flow was estimated by time-averaging the pulsed Doppler velocity signals obtained from a mid-artery sample volume. Then, the cuff was inflated to at least 50 mm Hg above systolic pressure to occlude arteries for 5 min and released abruptly. A mid-artery pulsed Doppler signal was obtained immediately on cuff release and no later than 15 s after cuff deflation to assess hyperaemic velocity. Post-occlusion diameters were obtained at 60, 80, 100 and 120 s after deflation. Endothelium-dependent FMD was calculated as the maximal postocclusion diameter relative to the averaged preocclusion diameters. At least 10 min of rest was given after the reactive hyperaemia before another image was acquired in order to reflect the re-established baseline conditions. Diameter measurements were measured at least three times at 3 to 4 min intervals after 0.6 mg sublingual nitroglycerine administration. The maximal nitroglycerine-mediated vasodilation (NMD) diameters were determined as the average of the three consecutive maximal diameter measurements after nitroglycerine use. The endothelium-independent NMD was then calculated as the percent change in diameter compared with baseline. All measurements of endothelial function were performed by an experienced operator who was blinded to all clinical data.

Measurement of high-sensitive C reactive protein

All subjects underwent blood sampling before endothelial function measurement. After a 12 h overnight fasting, all patients had a venous blood sample taken for measurement of high-sensitive C reactive protein (hsCRP). The blood samples were centrifuged at 3000 rpm for 10 min immediately after collection, and then the serum samples were kept frozen at

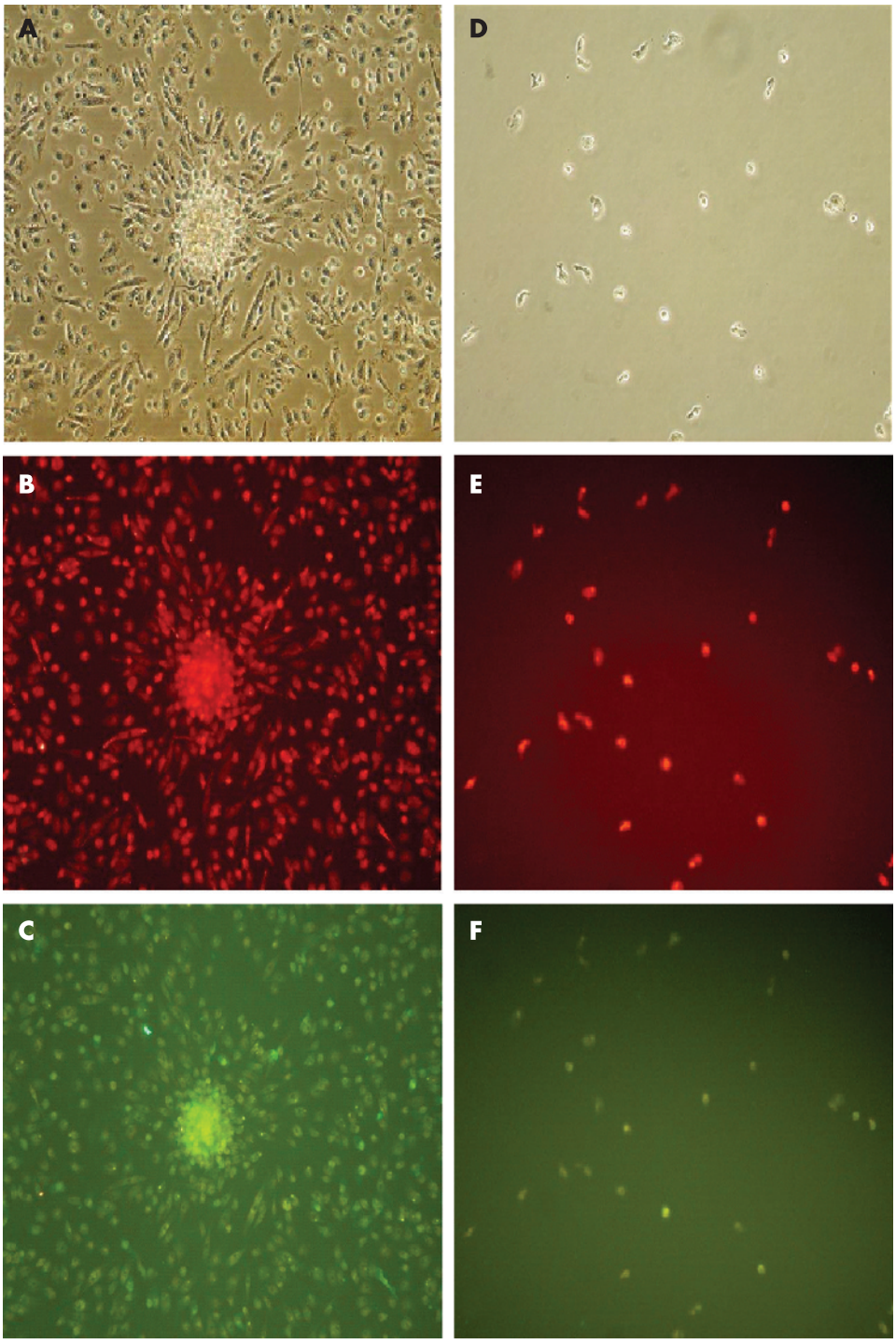

Figure 1 Phenotyping of endothelial characteristics of the colony-forming units of endothelial progenitor cells (A–C) and adhesive function assessed by fibronectin adhesive assay by indirect immunostaining with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-acetylated low-density lipoprotein (Dil-acLDL) and Bandeiraea simplicifolia lectin I (BS-1 lectin; D–F). A colony of endothelial progenitor cells was defined as a central core of round cells with elongated sprouting cells at the periphery (A–C). Most cells for adhesion function test were dual positive by indirect immunostaining with Dil-acLDL and BS-1 lectin (D–F).

−70℃ until analysis. Determination of hsCRP level was performed using latex-enhanced immunophelometric assay (Dade Behring, Marburg, Germany). All procedures were carried out according to the instructions of the manufacturers. Each standard and each serum sample were analysed two times, and the mean value was used for subsequent analysis.

Statistical analysis

All data were expressed as mean (SD) for numeric variables and as number (percentage) for categorical variables. A p value <0.05 was considered significant. Differences in baseline characteristics of underlying diseases, exercise habits, family history and treatments were compared using χ^2 test. Comparison among the three groups on continuous variables was made using analysis of variance test for normally distributed variables. Degree of association among independent variables such as age, sex, hypertension, serum lipid profiles, fasting glucose, systolic blood pressure, diastolic blood pressure, CFU numbers of EPC and FMD was measured by means of simple linear regression and multiple regression analyses. The SPSS 9.0 V.12 software package was used for statistical analysis.

RESULTS

Patients' baseline and exercise characteristics

A total of 34 subjects (48–73 years, mean (SD) age: 62 (7) years) were enrolled in the study, including 10 normal controls (60 (5) years, 6 women), 12 with CSX (63 (7) years, 6 women) and 12 with CAD (64 (9) years, 5 women). Table 1

Table 1 Baseline characteristics and drug use of 34 studied subjects in three groups

	Controls (n = 10)	CSX (n = 12)	CAD (n = 12)	p Values
Age (years)	60 (5)	63 (7)	64 (9)	0.260
Female	6 (60%)	6 (50%)	5 (42%)	0.772
Body mass index (kg/m^2)	25 (2)	25 (2)	26 (2)	0.155
Framingham risk score (%)	4 (4)	9 (7)	13 (9)	0.049
Systolic blood pressure (mm Hg)	126 (6)	129 (7)	134 (10)	0.060
Diastolic blood pressure (mm Hg)	78 (5)	79 (5)	77 (5)	0.619
Pulse pressure (mm Hg)	48 (6)	49 (8)	57 (9)	0.059
Left ventricular ejection fraction (%)	59 (4)	58 (4)	57 (4)	0.807
Exercise habits	4 (40%)	2 (17%)	2 (17%)	0.406
Family history of CAD	2 (20%)	2 (17%)	2 (17%)	1.000
Systemic hypertension	2 (20%)	10 (83%)	11 (92%)	0.001
Lipid profile (mg/dl)				
Total cholesterol	198 (26)	191 (24)	186 (30)	0.587
Triglycerides	115 (22)	131 (52)	142 (52)	0.399
High-density lipoproteins	56 (10)	49 (10)	40 (12)	0.005
Low-density lipoproteins	120 (28)	123 (21)	124 (22)	0.946
Fasting glucose (mg/dl)	89 (11)	93 (12)	103 (20)	0.098
Creatinine (mg/dl)	0.8 (0.2)	1.1 (0.4)	1.0 (0.2)	0.157
Medication use				
Angiotensin-converting enzyme inhibitors	0 (0%)	3 (25%)	1 (8%)	0.174
Angiotensin II receptor blockers	0 (0%)	4 (33%)	4 (33%)	0.113
Statins	1 (10%)	2 (12%)	4 (12%)	0.370

CAD, coronary artery disease.
Values are mean (SD) or number (%).

presents their baseline characteristics. There were no significant differences among the three groups regarding age, sex, body mass index, systolic blood pressure, diastolic blood pressure, pulse pressure, left ventricular ejection fraction, exercise habits, family history of CAD, and serum levels of total cholesterol, triglycerides, low-density lipoproteins and creatinine. More hypertension and lower high-density lipoproteins ($p<0.05$) were noted in the CSX and CAD groups than in normal controls. In addition, calculated Framingham risk score for estimating the10-year risk of developing coronary heart disease was significantly higher in patients with CSX and CAD than in normal controls ($p = 0.049$). There were no significant differences in drugs used, including angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers and statins.

Endothelium function, inflammation and circulating EPCs

As shown in table 2 and fig 2A, patients with CSX and CAD were found to have significantly decreased endothelial-dependent FMD compared with controls (controls vs CSX vs CAD: 10.6% (3.5%) vs 6.1% (1.8%) vs 4.1% (1.9%), $p<0.001$), but no difference was noted in endothelium-independent NMD (controls vs CSX vs CAD: 12.6% (3.9%) vs 11.9% (2.9%) vs 9.9% (3.8%), $p = 0.159$). Levels of hsCRP tended to be higher in patients with CSX and CAD than in control subjects, but the difference did not reach statistical significance (0.6 (0.5) vs 0.9 (0.5) vs 1.3 (0.8) mg/dl, $p = 0.065$).

Peripheral-blood mononuclear cells formed distinct colonies on fibronectin-coated dishes, as illustrated in fig 1A. As shown in table 2 and fig 2B, the numbers of EPC CFU were significantly reduced in subjects with CSX and CAD compared with normal controls (control vs CSX vs CAD: 41 (9) vs 30 (7) vs 14 (7) CFU/well, $p<0.001$). As illustrated in fig 3, the adhesive function assessed by fibronectin adhesion assay was shown to be impaired in patients with CSX and CAD compared with those in the control group (controls subjects vs CSX vs CAD: 104 (12) vs 80 (20) vs 65 (13) cells/well, $p<0.001$). Subjects with normotension had more EPC CFU numbers than those with hypertension (normotension vs hypertension: 34 (13) vs 24 (13) CFU/well, $p = 0.028$). A significantly attenuated EPC fibronectin adhesion function was also found in patients with hypertension compared with those without hypertension (normotension vs hypertension: 100 (17) vs 73 (18) cells/well, $p<0.001$). Patients $\geq$65 years were found to have a trend of having lower EPC CFU numbers than those <65 years (age $\geq$65 vs <65 years: 32 (14) vs 23 (11) CFU/well, $p = 0.067$).

Table 2 Comparison of baseline diameters of brachial artery, mean percent changes of diameter in response to flow-mediated vasodilation, nitroglycerine-mediated vasodilation, endothelial progenitor cell colony-forming units and high-sensitive C reactive protein in three groups of patients

	Controls (n = 10)	CSX (n = 12)	CAD (n = 12)	p Values
Baseline diameter (mm)	3.2 (0.6)	3.3 (0.3)	3.5 (0.6)	0.351
Maximal diameter (mm)	3.5 (0.7)	3.5 (0.4)	3.6 (0.6)	0.870
Flow-mediated vasodilation (%)	10.6 (3.5)	6.1 (1.8)	4.1 (1.9)	<0.001
Flow change during hyperaemic phase (%)	361 (96)	250 (70)	268 (152)	0.045
Baseline diameter before nitroglycerine-mediated vasodilation (mm)	3.2 (0.6)	3.3 (0.4)	3.5 (0.7)	0.496
Nitroglycerine-mediated vasodilation (%)	12.6 (3.9)	11.9 (2.9)	9.9 (3.8)	0.159
Endothelial progenitor cell colony-forming units	41 (9)	30 (7)	14 (7)	<0.001
Endothelial progenitor cell adhesion function (cells/well)	104 (12)	80 (20)	65 (13)	<0.001
High-sensitive C–reactive protein (mg/dl)	0.6 (0.5)	0.9 (0.5)	1.3 (0.8)	0.065

Values are mean (SD).

Correlation between levels of EPCs and endothelium-dependent FMD

As shown in table 3, fasting blood sugar and high-density lipoproteins were significantly associated with endothelium-dependent FMD ($r = -0.369$, $p<0.05$; $r = 0.368$, $p<0.05$, respectively). Furthermore, simple linear regression analysis on all studied subjects showed that levels of EPC CFU were significantly related to endothelium-dependent FMD ($r = 0.557$, $p = 0.001$, fig 4A) and high-density lipoproteins ($r = 0.339$, $p = 0.049$). Patients with higher cardiovascular risk assessed by Framingham risk scores have been found to be associated with decreased levels of EPCs ($r = -0.332$, $p = 0.055$), although the difference did not reach statistical significance (fig 4B). When all univariate baseline parameters were entered into a multiple regression analysis, the results showed that numbers of EPC CFU were different in normal controls, subjects with CSX and stable patients with CAD ($p<0.01$), and were also significantly related to FMD ($p<0.001$).

Effect of tumour necrosis factor α on circulating EPCs

We tested the effects of tumour necrosis factor α (TNFα) at different concentrations on circulating EPCs cultured from patients with CSX. After seeding mononuclear cells (1×10^7) on a fibronectin-coated six-well plate and changing the medium on day 4, adherent cells were incubated with different concentrations of TNFα (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). As shown in fig 5, TNFα dose-dependently suppressed numbers of EPCs (5, 10, 15 and 20 ng/ml). Similar results were observed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (data not shown). Interestingly, pretreatment with simvastatin for 24 h (1 and 5 μM) reversed the suppression effect of TNFα on EPCs. These findings may explain the beneficial effects of statins in treating patients with CSX by increasing EPC levels.

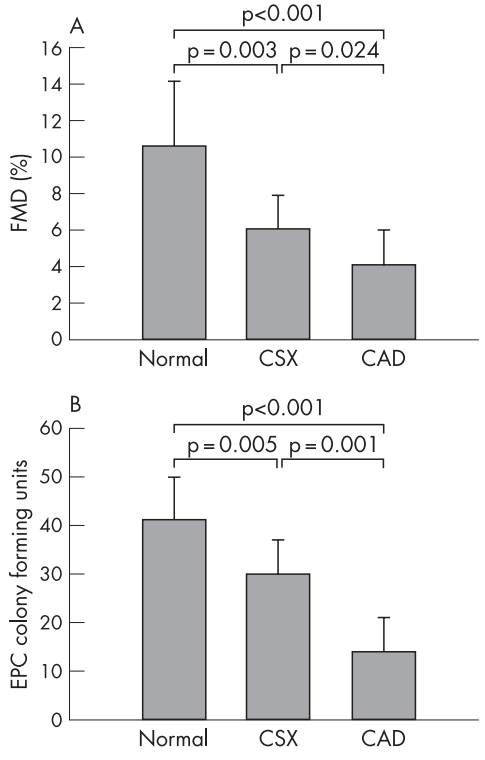

Figure 2 Comparison of percent changes of endothelium-dependent flow-mediated vasodilation (FMD; A) and numbers of endothelial progenitor cell (EPC) colony-forming units (B) in three groups of subjects. CAD, coronary artery disease; CSX, cardiac syndrome X.

DISCUSSION

To the best of our knowledge, this is the first study to show decreased levels and adhesive function of circulating EPCs in patients with CSX, suggesting that altered EPC biology may contribute to endothelial dysfunction and microvascular abnormalities observed in CSX. In addition, TNFα dose-dependently suppressed EPC numbers, and this suppression effect was abolished by pretreatment with simvastatin. These findings may explain the benefit of statins in treating patients with CSX by increasing EPC levels.

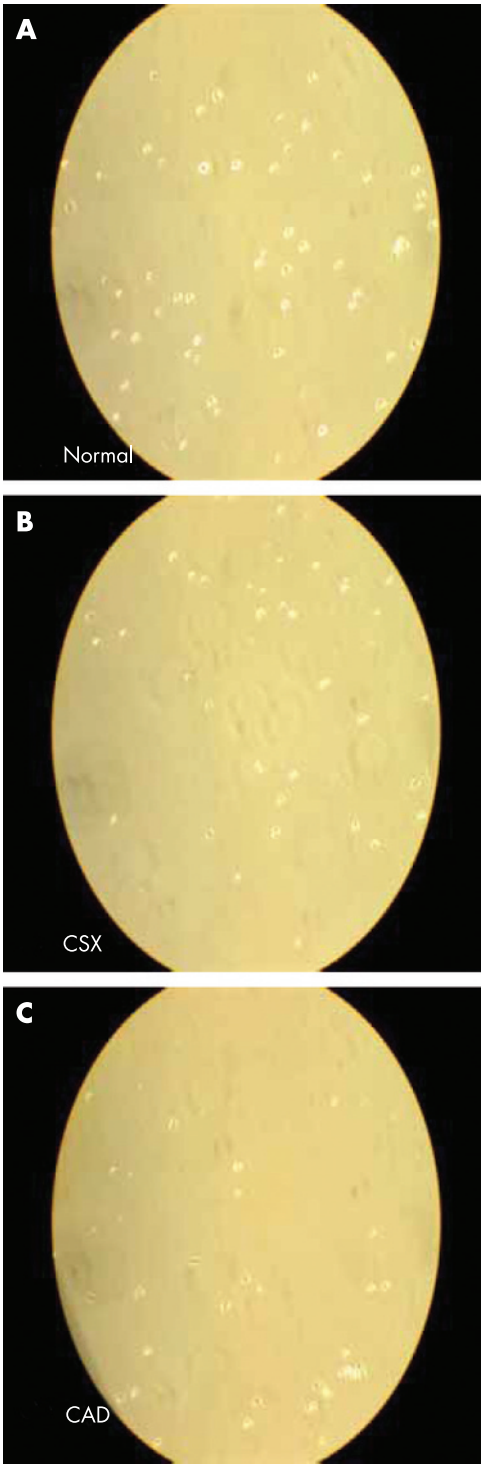

Figure 3 Endothelial progenitor cell adhesive function assessed by fibronectin adhesion assay in normal controls (A), subjects with cardiac syndrome X (CSX; B) and coronary artery disease (CAD; C).

Table 3 Simple linear regression analysis for determinants of endothelial progenitor cell colony-forming units

Variables	r	p Value
Age	−0.281	0.108
Framingham risk scores	−0.332	0.055
hsCRP (mg/dl)	−0.277	0.112
Flow-mediated vasodilation (%)	0.557	0.001
Systolic blood pressure (mm Hg)	−0.235	0.182
Diastolic blood pressure (mm Hg)	0.174	0.325
Pulse pressure (mm Hg)	−0.330	0.057
Body mass index	−0.254	0.147
Total cholesterol (mg/dl)	0.007	0.970
Triglycerides (mg/dl)	−0.221	0.210
High-density lipoproteins (mg/dl)	0.339	0.049
Low-density lipoproteins (mg/dl)	−0.144	0.417
Fasting glucose (mg/dl)	−0.224	0.204
Creatinine	−0.123	0.488

hsCRP, high-sensitive C reactive protein.

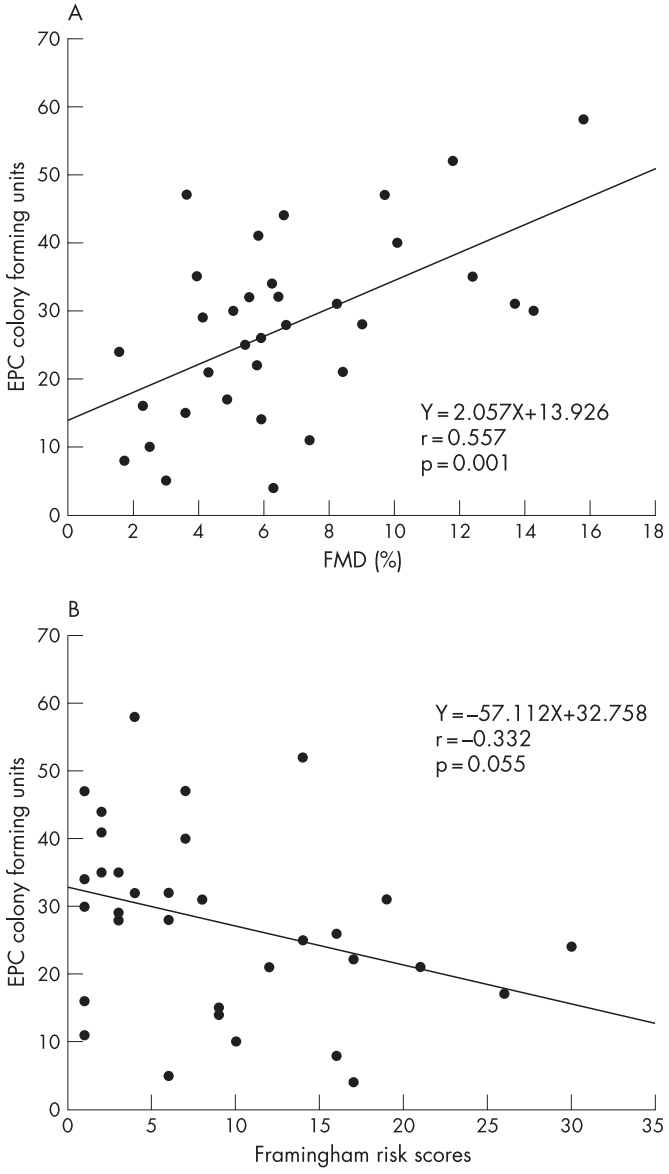

Figure 4 Algorithm showing distribution of endothelial progenitor cell (EPC) colony-forming units, and endothelium-dependent flow-mediated vasodilation (FMD; A) and Framingham risk scores (B) in total 34 subjects.

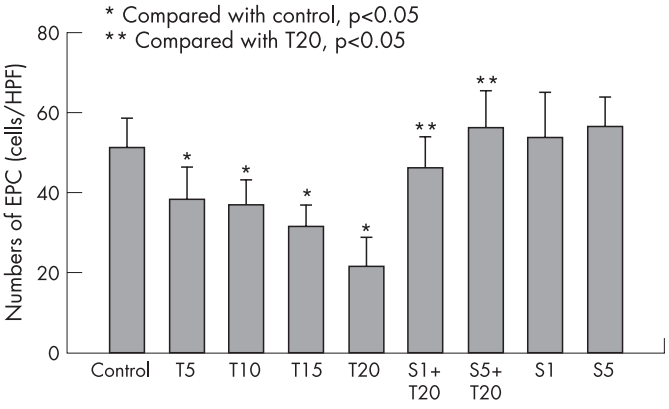

Figure 5 Endothelial progenitor cells (EPCs) were isolated and incubated with different concentrations of tumour necrosis factor α (TNFα) for 72 h. EPCs were characterised as adherent cells that were dual positive for lactin staining and DiI-acLDL uptake. The numbers of EPCs were counted under a microscope, and data are expressed as mean numbers of EPCs per high-power field (HPF; SD). T: TNFα, T5: 5 ng/ml, T10: 10 ng/ml; T15: 15 ng/ml, T20: 20 ng/ml; S: simvastatin, S1: 1 μM, S5: 5 μM.

Although CSX has been proposed for more than three decades, the underlying mechanisms in CSX have been shown to be diverse and remain unclear. Factors including reduced coronary flow reserve, abnormal pain perception, impaired endothelial function, altered adrenergic activity, autonomic dysfunction, increased platelet aggregability and early stages of cardiomyopathy have been proposed as the possible mechanisms of CSX. However, the most convincing evidence suggested that microvascular ischaemia secondary to generalised endothelial dysfunction is a leading pathophysiological explanation for CSX.[6 11] Endothelial function in patients with CSX has been shown to be similarly impaired as in patients with established CAD,[11] and about 60% of patients with CSX with proved coronary endothelial dysfunction developed CAD after long-term follow-up.[20]

During the past decades, a large body of evidence accumulated has indicated that the endothelium is a barrier between blood and vessel wall and also provides essential vasculoprotective functions. The functional activity and integrity of the endothelial monolayer play a pivotal role in atherogenesis. The extent of endothelial injury may represent a balance between the magnitude of injury and the capacity for repair. Traditional view suggests that endothelium integrity is maintained by neighbouring mature endothelial cells which migrate and proliferate to restore the injured endothelial cells. However, a series of clinical and basic studies prompted by the discovery of bone marrow-derived EPCs have provided new insights into these processes and suggest that the injured endothelial monolayer is regenerated partly by circulating bone marrow-derived EPCs.[21] Levels of circulating endothelial progenitor cells have been shown to be associated with endothelial function and cardiovascular risk factors, and help to identify patients at increased cardiovascular risk.[15] Reduced levels of circulating EPCs independently predict atherosclerotic disease progression and development of cardiovascular events, thus supporting an important role for endogenous vascular repair of EPCs in modulating the clinical course of CAD.[17 18] However, no previous literature has reported on the role of circulating EPCs in patients with CSX. In the present study, patients with CSX, although with normal coronary angiography, have been found to have decreased levels and adhesion function of circulating EPCs, which may contribute to coronary endothelial dysfunction and microvascular ischaemia. These results suggest that attenuated endothelial regeneration capacity might play a critical role in the pathophysiological mechanisms of CSX, and should be considered a therapeutic target in treating these patients.

Systemic inflammation, and its association with endothelial dysfunction, has proven to play a key role in atherogenesis. Some clinical evidence has indicated that patients with CSX have increased inflammation when compared with controls.[22] The level of C reactive protein in patients with chest pain and normal coronary arteries correlates with the frequency and duration of chest pain and the extent of ST-segment depression on exercise testing and ambulatory monitoring.[23] Verma *et al*[24]reported that recombinant human CRP directly inhibits EPC differentiation, survival and function at concentrations known to predict adverse vascular outcomes. Therefore, the enhanced extent of inflammation observed in patients with CSX may suppress EPC levels and function in blood circulation, resulting in attenuated repair capacity of vasculature. our data supported these findings, which showed a significant dose-dependent suppression effect of TNFα on EPCs. Moreover, the inhibitory effect of TNFα on EPCs was reversed by pretreatment with simvastatin, supporting previous reports that statin therapy can exert pleiotropic effects and result in improvement on both endothelial function and exercise-induced ischaemia in CSX.[25 26] These beneficial results may be due to the contribution of bone marrow-derived EPCs, which are mobilised in response to statin therapy and subsequently improve endothelial function.[27 28]

Study limitations

The study has some limitations which should be considered. First, the study population is relatively small. These data, however, are based on a careful selection of patients, thus limiting the enrolment of large numbers. Second, a pharmacological stimulation test was not performed in excluding vasospasm in patients with CSX owing to ethical consideration. Third, an intravascular ultrasound was not carried out at baseline assessment. Therefore, it is unknown whether any of the patients with CSX had atherosclerosis not identifiable by coronary angiography, and whether this contributed to occult CAD despite normal coronary angiography.

CONCLUSIONS

In the present study, similar to the patients with CAD, decreased numbers and adhesive function of EPCs were also found in patients with CSX. These findings provide new evidence which may explain the underlying pathophysiological mechanisms that are responsible for the endothelial dysfunction and microvascular abnormalities observed in patients with CSX.

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Competing interests: None declared.

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